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Oxidative stress and muscle homeostasis

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Purpose of review

The term oxidative stress is often used to indicate a condition in which the accumulation of reactive oxygen species is considered just damaging. We will discuss both the physiological and pathological role of oxidative stress on skeletal muscle homeostasis and function, and how oxidative stress can activates opposite signaling molecule to regulate gene and protein expression to guarantee muscle adaptation and to trigger a pathological condition.

Recent findings

Emerging evidences have assigned a critical role to oxidative stress in muscle homeostasis and in the physiopathology of skeletal muscle, suggesting that reactive oxygen species are not merely damaging agent inflicting random destruction to the cell structure and function, but useful signaling molecules to regulate growth, proliferation, differentiation, and adaptation, at least within physiological concentration.

Summary

The role of oxidative stress on muscle homeostasis is quite complex. It is clear that transiently increased levels of oxidative stress might reflect a potentially health promoting process, whereas an uncontrolled accumulation of oxidative stress might have pathological implication. Additional work is, therefore, necessary to understand and define precisely whether the manipulation of the redox balance represents a useful approach in the design of therapeutic strategies for muscle diseases.

Keywords

muscle adaptation, muscle atrophy, reactive oxygen species

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Introduction

Homeostasis represents one of the most important and critical parameters of adult skeletal muscle and it is defined as the capability of a system to maintain a constant state of complexity and order in a dynamic equilibrium. How oxidative stress contributes to guarantee or to alter this internal balance is still an open issue. To date, the term oxidative stress is frequently used to define only a 'pathological' condition in which the production of reactive oxygen species (ROS) is just considered damaging [1]. However, emerging evidences suggest that ROS can act also as important signaling molecules in muscle contraction and adaptation. Yet, the effects of ROS are dose dependent, and at high levels, these highly reactive molecules exert toxic effects on the cell and invoke profound changes in gene expression. This suggests that there is a threshold at which a physiological effect of ROS turns into a pathological one.

The physiological role of oxidative stress on skeletal muscle

Generation of ROS represents one of the most prominent events during contractile activity, suggesting that it could influence muscle function and health [2,3]. How do ROS act as physiological signaling molecules? When does the oxidative stress become a damaging factor?

It is well recognized that moderate, nonexhaustive physical exercise has beneficial effects and might prevent several chronic diseases [4•,5,6] (Fig. 1). A critical step in the activation of the adaptive response is the crosstalk between mitochondria and Ca^{2+} [7•]. Interestingly, electron microscopy and electron tomography analysis [8••] identified small bridges, or tethers, that link the outer mitochondrial membrane to the intracellular Ca^{2+} stores, confirming previous studies [9,10] and supporting a morphological model of the functional crosstalk between mitochondria and calcium stores of muscle. The mechanism of this reciprocal organelle docking remains unresolved but it has been proposed that it depends on the expression on both membranes of complementary proteins that link the two organelles together, possibly at specific sites [11].

Mitochondria are the major source of ROS production [12]. In turn, these organelles respond to elevated ROS generation by undergoing morphological and functional adaptations. Exercise that increased ROS production

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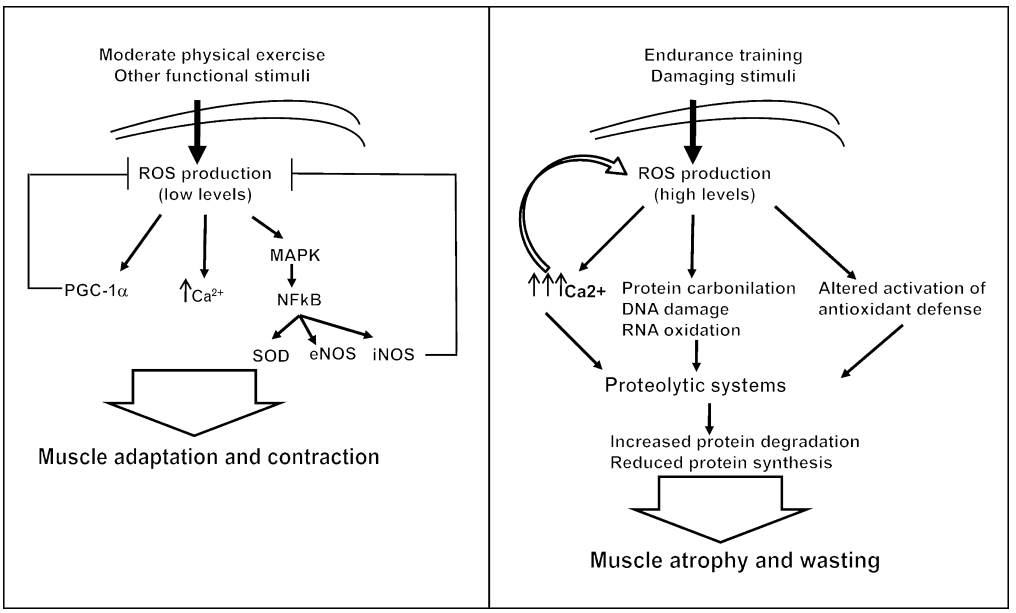
Figure 1 A summary of the role of oxidative stress in the physiopathology of skeletal muscle



Left panel illustrates the physiological role of oxidative stress and indicates the potential mechanisms involved in muscle contraction and adaptation. Moderate, nonexhaustive physical exercise induces the production of ROS, which in turn activates specific molecules (PGC-1α, MAPK, and antioxidant enzymes) that function as regulators of intracellular ROS levels. Thus, low levels of ROS have beneficial effects and might prevent several chronic diseases. Right panel illustrates the pathological effect of oxidative stress. Endurance training, damaging stimuli, or both deregulate the activation and function of important intracellular mechanisms of defense against the accumulation of free radicals. Thus, the accumulation of ROS promotes the activation of proteolytic systems, with the consequence of an increase in protein degradation and reduction in protein synthesis, leading to muscle atrophy and wasting. eNOS, endothelial nitric oxide synthase; iNOS, inductible nitric oxide synthase; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappa B; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; ROS, reactive oxygen species; SOD, superoxide dismutase.

stimulates peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) expression [13], a critical factor involved in mitochondriogenesis and that represents an important regulator of intracellular ROS levels [14] (Fig. 1). More recently, it has also been reported that the physiological expression of the *PGC-1α* gene requires an optimal concentration of ROS [15]. Low levels of ROS result in reduced PGC-1α mRNA. In contrast, elevated levels of ROS induce PGC-1α transcription indirectly, via AMP-activated protein kinase activation [15]. This should guarantee the activation of a controller mechanism with the purpose to maintain the ROS production at physiological levels (Fig. 1). However, the physiopathological role, if any, of these differences is not established yet.

Ca^{2+} is a critical component of muscle contraction and its intracellular concentration must be finely regulated [16]. Notably, several Ca^{2+} transport systems are modulated by oxidation, which increases the activity of inositol (1,4,5)-triphosphate (IP3) and ryanodine receptors (RyRs), the main intracellular channels releasing Ca^{2+}. Thus, in a physiological context, nitric oxide and ROS are produced in contracting muscle and have been shown *in vitro* to modulate the RyRs' redox state and channel activity [17,18]. In particular, it is well established that oxidation of critical RyR thiol groups activates the Ca^{2+} release mechanism, whereas addition of thiol-reducing agents close down the Ca^{2+} channel [18]. Thus, modulation of redox potential of reactive thiols may be a general control mechanism by which sarcoplasmic/endoplasmic reticulum, RyR/IP3 receptors, and mitochondria control cytoplasmic Ca^{2+} concentrations. However, this process must be regulated to guarantee a physiological process. It has been, in fact, demonstrated that enhanced Ca^{2+} leak from mutant RyR1 Ca^{2+}-release channels increases oxidative stress, leading to *S*-nitrosylation of RyR1 that further enhances Ca^{2+} leak and increases susceptibility to heat-induced sudden death [19••] (Fig. 1).

Oxidative stress, muscle adaptation, and molecular mechanisms

What are the molecular mechanisms involved in muscle adaptation?

There is growing evidence demonstrating that along the activation of PGC1-α, low concentrations of ROS induce the expression of antioxidant enzymes and other defense mechanisms. It has been recently reported that exercise causes an activation of mitogen-activated protein kinases, which in turn activates the nuclear factor kappa B

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pathway and consequently the expression of important enzymes associated with defense against ROS (superoxide dismutase or SOD) and adaptation to exercise [endothelial nitric oxide synthase (NOS) and inductible NOS] [20] (Fig. 1). Additional cellular components sensitive to redox changes and critical component of adaptation are activator protein-1, heat shock transcriptional factor-1, insulin receptor kinase, and protein tyrosine phosphatases [21].

Noteworthy, the beneficial effects of exercise are abolished by administration of antioxidant compounds such as vitamins C and E [20,22•,23••]. The adverse effects of antioxidant treatment suggest that ROS act as critical signals in exercise because their decreasing formation prevents activation of important signaling pathways that cause useful adaptations in muscle [24].

Nevertheless, the beneficial effects of exercise are lost with exhaustion (Fig. 1, right panel). This can be explained considering that endurance exercise of extreme duration and extreme intensity under extreme conditions, such as hypoxia, generates much higher levels of free radicals that overwhelm cellular antioxidant defenses, and cause tissue damage [25,26].

The opposite effects exerted by different concentration of ROS can be justified considering the concept of hormesis [27], in which a low dose of a substance is stimulatory and a high dose is inhibitory. Thus, muscle benefits from low doses of radicals, whereas it is damaged by exposure to high levels of these radicals.

Oxidative stress as signal molecules regulating stem cell fate

It is well accepted that a low concentration of ROS influences cell growth, differentiation, and proliferation. Notably, it has been recently reported that ROS can also act as signal molecules to instruct the fate of uncommitted muscle cells. In particular, mitochondrial ROS production has been implicated in the adipogenic conversion of muscle satellite cells [28•]. These data further add complexity into the physiopathological signaling activated by ROS, as they suggest that transdifferentiation of mesenchymal precursors into adipocytes may play a primary pathogenic role in muscle aging.

Thus, clarifying the molecular basis of ROS-mediated signals in cell adaptation and differentiation is crucial to design novel therapeutic approaches.

The pathological role of oxidative stress on skeletal muscle

A crucial system severely affected in different pathological conditions is the antioxidative defense, leading to accumulation of ROS (Fig. 1). The discovery that the antioxidant status decreases with age and it is affected in several pathological conditions, such as disuses, chronic fatigue syndrome, liver and kidney diseases, cancer, muscular dystrophy, and amyotrophic lateral sclerosis (ALS), has placed oxidative stress as a central mechanism in the pathogenesis of these diseases [3]. However, how such an oxidative insult plays a direct role in the disease-related decrease of muscle performance and mass (atrophy) remains largely unknown. In addition, the discrepancy among different studies has further complicated the achievement of a conclusive link between altered balance of ROS generation and atrophy-associated diseases.

Muscle disuse is a common pathological condition as an integral part of several diseases, leading to muscle atrophy. It has been reported that immobilization is associated with an increase in protein carbonylation, protein degradation, and a reduction in protein synthesis [29–31]. Additionally, denervated atrophic muscles exhibit a significant peroxidation of the membrane network, suggesting a toxic effect of excessive ROS production [32]. Moreover, other studies [33–36] have highlighted the role of oxidative stress in atrophic muscle due to an unbalance between the cellular antioxidant systems and ROS production.

These data were supported by a proteomic approach [37] performed on mice hindlimb unloaded, an experimental model of muscle atrophy. In this study, were compared the protein profile of soleus, a slow oxidative muscle, and gastrocnemius, a fast glycolitic muscle, of both control mice and of mice hindlimb-unloaded for 14 days [37]. The protein pattern of soleus and gastrocnemius showed large differences in adaptation to hindlimb unloaded, which comprised the antioxidant defense systems and stress proteins, myofibrillar proteins, energy production systems, transport proteins, and several other proteins having a variety of functional roles [37]. In particular, antioxidant defense systems were impaired in hindlimb-unloaded soleus and enhanced in hindlimb-unloaded gastrocnemius, consistently protein oxidation index and lipid peroxidation were higher in hindlimb-unloaded soleus but normal in hindlimb-unloaded gastrocnemius [37]. Noteworthy, both soleus and gastrocnemius muscles were atrophic [37]. These data confirm the evidence that hindlimb unloaded induces muscle atrophy and suggest that different muscles activate, in a different manner, the defense systems against oxidative stress.

In other studies, it has been demonstrated that iron, which accumulates during aging, accelerates the production of ROS [38•] that in turn damage not only proteins and DNA but also RNA [39•]. The higher susceptibility of RNA, compared with DNA, to oxidative

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damage could be due to a greater exposure of RNA to ROS and iron, or to differences in protection from ROS, repair, or turnover mechanisms. Thus, therapeutic interventions that counteract intracellular iron accumulation or that can increase the control of iron (such as chelators) in skeletal muscle may be especially beneficial in attenuating disuse-induced muscle atrophy in aged animals.

However, whether oxidative stress is a requirement or merely plays a regulatory role in the progression of muscle atrophy is still an open question.

The critical role of oxidative stress on muscle atrophy

A key question that remains to be addressed is the following: does oxidative stress alone trigger muscle atrophy or is ROS-mediated oxidative stress a consequence of muscle atrophy? In addition, another important issue to address is whether a selective accumulation of oxidative stress in skeletal muscle is sufficient to induce and promote systemic effects.

A clear answer is still missing. Nevertheless, recent reports contribute to clarify how the disruption of the delicate balance between ROS production and antioxidant defense may activate a cascade of events, leading to muscle atrophy and wasting.

SOD enzymes are antioxidants that protect cells from oxidative stress by catalyzing the dismutation of superoxide to oxygen and hydrogen peroxide. It has been previously reported that SOD2 knockout mice exhibit reduced electron transport chain components and lipid accumulation in skeletal muscle [40,41]. Additionally, SOD2 knockout mice display a dilated cardiomyopathy [40,42]. These studies, however, did not explicitly address the importance of SOD2 function in the muscle for whole organism vitality and longevity. Martin *et al.* [43•] extended these previous reports, demonstrating that the selective knockdown of SOD2 in the musculature of *Drosophila* is sufficient to shorten life span and to accelerate locomotor declines. Flies with knockdown of SOD2 in muscle exhibit mitochondrial disorder, reduced ATP content, and elevated caspase activity, suggesting that the consequences of SOD2 loss in this tissue extend to the viability of the organism as a whole.

Another study by Zhang *et al.* [44] suggested other interpretations about the role of oxidative stress and longevity. It has been previously reported that the single knockout in two mitochondrial-localized antioxidant enzymes, Mn SOD (MnSOD) and glutathione peroxidase-1 (Gpx-1), displays altered mitochondrial function, increased sensitivity to apoptosis, increased cancer incidence, and susceptibility to oxidative stress, without significantly altering the life span [45–50]. Because the antioxidant defense system is a complex and integrated system, it is possible that deficiency of a single antioxidant enzyme may not compromise the system to a magnitude sufficient to alter longevity. To this purpose, the authors generate a double knockout model reporting that mice deficient in both MnSOD and Gpx-1 have increased oxidative damage and a greater incidence of disorder but no reduction in longevity [44].

These evidences suggest that altered balance of ROS production is a critical issue for the progression of the diseases and that pathological conditions in which oxidative stress is a common feature, such as aging and disuses, may not be simply an issue of living or dying, but rather an issue of functioning well versus functioning poorly [51].

Our recent studies [52••,53•] further provide important new insights concerning the atrophic effect of oxidative stress on muscle phenotype, demonstrating that skeletal muscle is a direct target of the toxic properties of a mutant SOD1 variant ($SOD1^{G93A}$) found in human familial ALS. When overexpressed exclusively in skeletal muscle, mutant $SOD1^{G93A}$ induces accumulation of ROS and sarcolemma damage, causes dramatic muscle atrophy with a concomitant alteration in the ultrastructure and in the functional performance of skeletal muscles, promotes a shift in the metabolic activity of muscle fibers, and activates proteolytic systems and autophagy–lysosome [52••,53•]. A similar phenotype has also been reported in other studies [54,55•], in which muscle-restricted overexpression of the mitochondria truncated form of the nuclear receptor TRa1, p43, induces an oxidative stress despite stimulation of antioxidant enzyme activities. This oxidative stress induces skeletal muscle atrophy detectable at 6 months of age, concomitant to an upregulation of the two muscle-specific ubiquitin ligases E3, atrogin-1/MAFbx and MuRF1 [55•].

These data would suggest that interfering with oxidative stress should rescue the atrophic or pathological phenotype. Accordingly, amelioration of disuse muscle atrophy following antioxidant administration has been observed [56–58]. Thus, a critical role of oxidative stress in muscle atrophy and wasting associated with disuse can be widely recognized. Nevertheless, the situation has been complicated by different studies comparing the muscle mass of animals treated with antioxidant compounds, reporting divergent results ranging from an absence of positive effect, to a rescue of atrophic phenotype, to an even negative effect.

Noteworthy, Brocca *et al.* [37] demonstrated that antioxidant administration prevented the impairment of the

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antioxidant defense systems in soleus and further enhanced them in gastrocnemius subjected to hindlimb unloaded. However, muscle atrophy was not prevented, suggesting that antioxidant supplementation is not an effective countermeasure to the atrophy associated with hindlimb and opening the possibility that oxidative stress might not be a major requirement of muscle atrophy also in other conditions.

A similar approach, but with different outcome, has been used in another experimental model of muscle atrophy, the MLC/SOD1^{G93A} mice, which develops progressive muscle atrophy, associated with a significant reduction in muscle strength, alterations in the contractile apparatus, and mitochondrial dysfunction [52••]. Transgenic mice treated intraperitoneally for 15 days, with 30 mg/kg of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma Aldrich), a cell-permeable water-soluble derivative of vitamin E with potent antioxidant properties [59,60], significantly reduced the toxic effect of ROS, partly rescuing muscle phenotype and the functional performance of transgenic muscle [52••].

As reported above, SOD2 knockdown in the musculature alone was sufficient to cause the shortened life span and accelerated locomotor declines [43•]. This result led the authors to propose that muscle overexpression of SOD2 enzyme might extend life span or preserve locomotor behavior across age.

Paradoxically, overexpression of SOD2 in muscle had no effect or possibly even negative effects [43•]. How do we explain this?

It is possible that overexpression of SOD2 beyond a level alters the delicate balance between ROS production and antioxidant defense. Because ROS can act as important signaling molecules, as reported in the previous paragraph, in addition to driving oxidative damage, any negative outcomes associated with SOD2 overexpression could be due to inhibition of ROS signaling.

This hypothesis has been validated by studies [20,22•,23••] in which the antioxidants treatment prevents health-promoting effects of physical exercise in humans. In particular, it has been demonstrated that ROS are required for cellular adaptations to exercise and for the insulin-sensitizing capabilities of physical exercise in healthy humans and that commonly used antioxidants, such as vitamins C and E, abrogate the health-promoting effects of physical exercise.

Conclusion

All of these findings demonstrate the complexity of oxidative stress in muscle homeostasis.

Nevertheless, it is clear that transiently increased levels of oxidative stress might reflect a potentially health promoting process, whereas an uncontrolled accumulation of oxidative stress might have pathological implication (Fig. 1).

Further studies are needed to fully elucidate the conditions under which oxidative stress is beneficial or detrimental for skeletal muscle.

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